Supporting Information

Coupling IR-MALDESI with Drift Tube Ion Mobility-Mass Spectrometry for High-Throughput Screening and Imaging Applications

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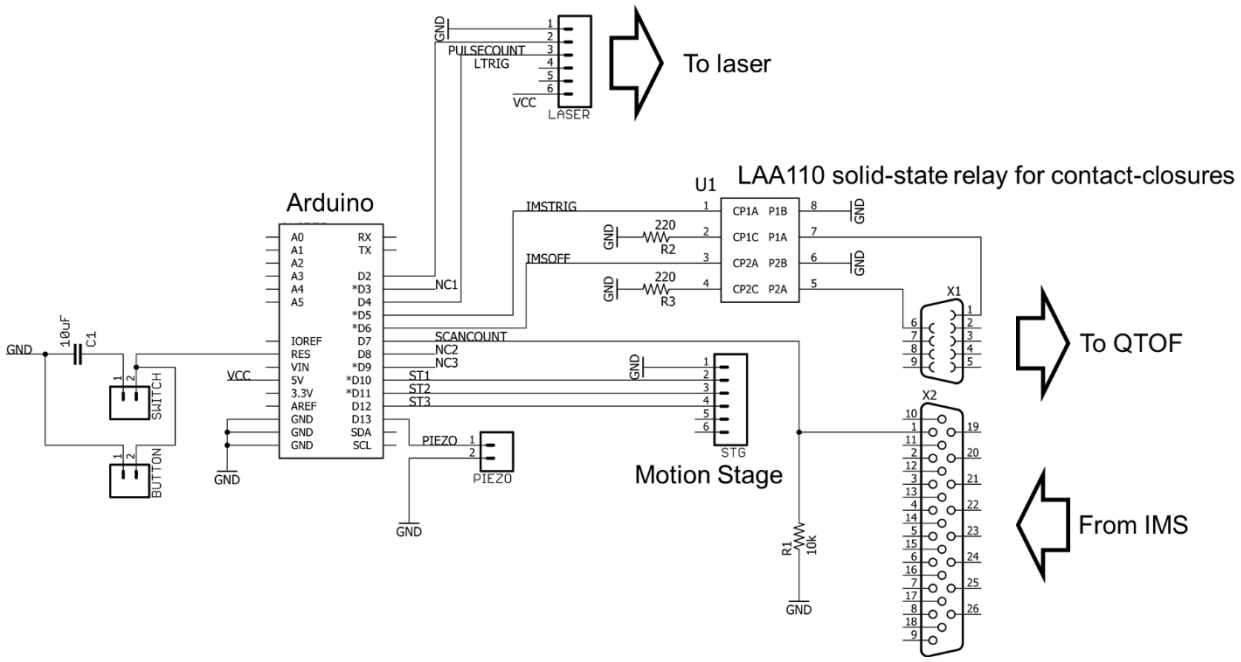
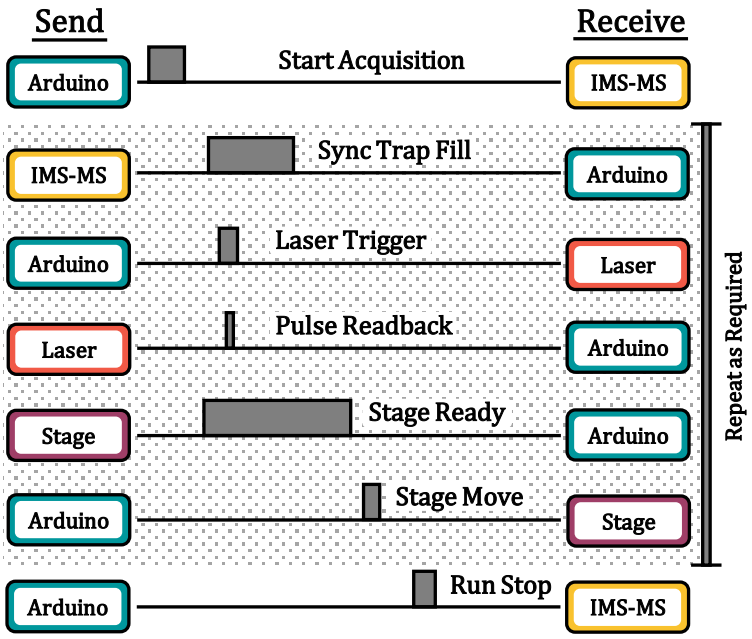
IR-MALDESI-IMS-MS Synchronization and Data Presented in this Work

In this supporting information we detail information for the instrumental setup used in this work. Extended data pertaining to the Coca-Cola analysis is provided, with both mass and mobility information as needed. An illustrative description of the communication flow between imaging components is also included. Lastly, additional mobility analysis for both the hexose sugars protein is provided.



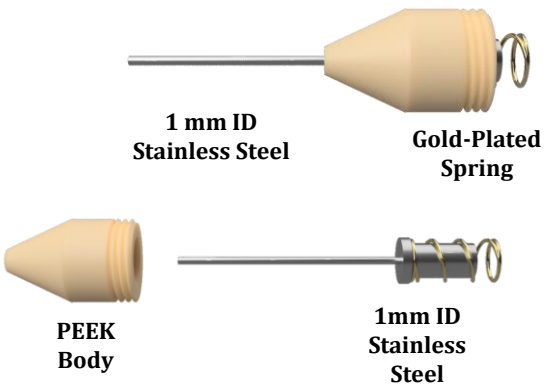
Supplemental Figure S1. Complete circuit diagram for triggering of each component in the developed IR-MALDESI interface coupled with the Agilent 6560 IMS-QTOF system.



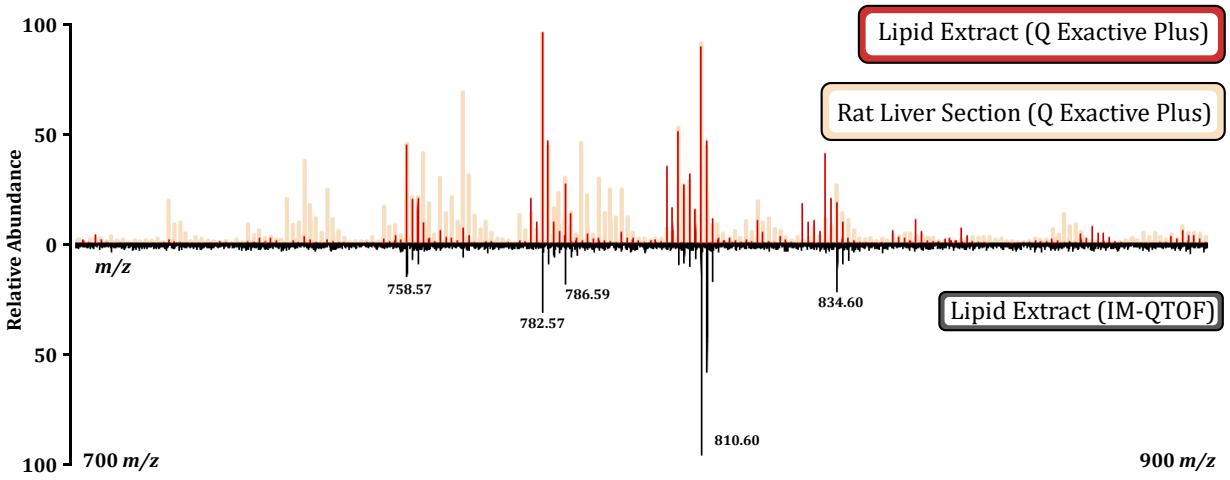
Supplemental Figure S2. Illustrative depiction of command flow for the IR-MALDESI-IMS-MS workflow. Boxes are not to scale. Trap Fill times used for the 6560 IMS-MS can vary, but in this work Trap Fill time was set to 40 ms. In a high-throughput workflow or imaging acquisition (*i.e.*, well plate or restoring experiment), the middle steps are repeated for each analyte spot until the experiment is complete.

Instrument Conditions	
Ion Polarity	+
Mobility Calibrants	Agilent Tune Mix
CCS Method	Single Field
Drift Gas	Nitrogen
Temperature	28 Celcius
Drift Tube Pressure	3.95 Torr
E/N	17 V/cm
Length of Tube	78 cm
Maximum Drift Time	60 ms
Trap Release Time	100 µs

Supplemental Figure S3. IMS-MS settings used for measuring drift times (and calculating CCS) in this experiment.



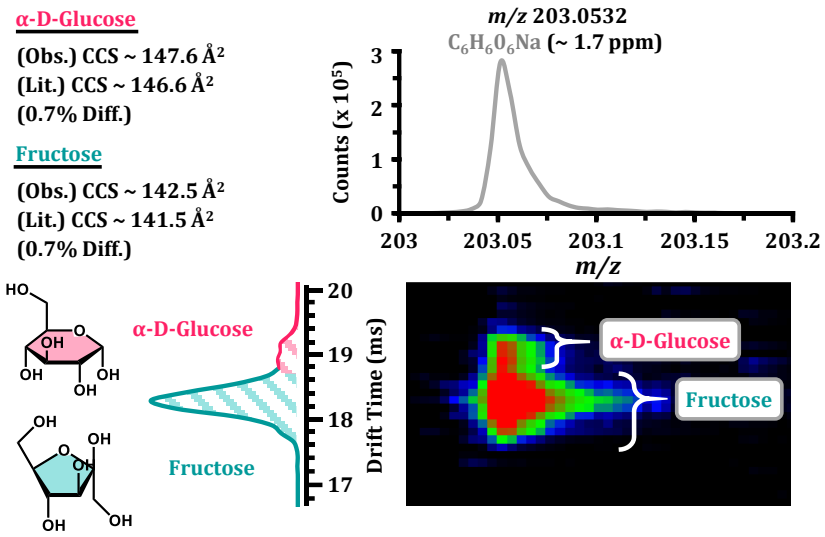
Supplemental Figure S4. Modified extended inlet designed for the IR-MALDESI system. The extended inlet designed here is necessary for adjustments in stage movement under the laser/ESI ionization region. The gold-plated spring used here is identical to that of the Agilent Nanospray source, along with the corresponding ID magnet used for source recognition override and defeating the safety door interlock on the source region.



Supplemental Figure S5. (Top) Lipid analysis of both fresh rat liver section (yellow analyzed directly - no extraction- *via* IR-MALDESI) and lipid extract from the same rat liver (red, directly infused) measured on the Q Exactive Plus mass spectrometer. **(Bottom)** Lipid extract spectra obtained on the IM-QTOF platform, with annotations as noted in **Figure 4** of the main text.

Annotation	m/z	Adduct	Single Field CCS (This Experiment)	Literature CCS	% Diff.
PC_34:2	758.57	$[M+H]^+$	283.8	279.5	-1.5
PC_34:1	760.58	$[M+H]^+$	287.0	282.0	-1.8
PC_34:1	782.57	$[M+Na]^+$	286.8	284.8	-0.7
PC_36:2	786.59	$[M+H]^+$	291.4	285.9	-1.9
PC_36:3	806.57	$[M+Na]^+$	288.5	287.0	-0.5
PC_36:1	810.6	$[M+Na]^+$	295.5	290.3	-1.8
PC_38:3	834.6	$[M+Na]^+$	296.9	293.1	-1.3

Supplemental Figure S6. Single Field CCS values for the PC lipids identified using the IR-MALDESI-IMS-MS platform described in the work. Comparisons to literature ("Literature CCS") are based on the findings of Leaptrot, K. L. *et. al.* (*Nature Communications*, **2019,** 10, 985). The lipids annotated in this work and in that of Leaptrot are not based on pure standards, and hence total lipid characterization (*e.g.* location and geometry of double bonds) cannot be stated. This may account for some of the CCS discrepancy noted between studies.



Supplemental Figure S7. Mobility separation for carbohydrate isomers subsequent to the analysis of **Figure 5D** of the main text. This experiment was used to calculate the CCS of each isomer using the single field method, as described by Stow *et al.* (*Anal. Chem.* **2017**, *89*, 9048-9055). The CCS values for each distribution agree to 0.7% CCS difference with the values previously measured on the same platform (*Anal. Chem.* **2018**, *90*, 14484-14492).

Lysozyme Charge States and CCS_{N2} ($Å^2$)

Charge State	Sullivan[1] (ESI 2017)	Shrestha[2] (LAESI 2014)	Curr. Study (ESI)	Curr. Study (IR-MALDESI)	% Diff. (Sull.) (vs. ESI)	% Diff. (Shrest.) (vs. ESI)	Curr. Study % Diff. (ESI vs. IR-MALDESI)
7^+	1600	1723	1694	1681	-5.7	1.7	0.8
8^+		2249	2097	2037		7.0	2.9
9^+		2375	2221	2192		6.7	1.3
10^+	2280	2323	2351	2353	-3.1	-1.2	-0.1

[1]Sullivan *et. al. Chem. Commun.* **2017**, 53, 4246-4249.

[2]Shrestha, B. and A. Vertes, *Anal. Chem.* **2014**, 86, 4308-4315.

Supplemental Figure S8. Consistent CCS values were obtained for lysozyme using both conventional ESI and IR-MALDESI. Both spectra were obtained in 50% organic for higher ionization efficiency. While these conditions do not reflect the "native" state, agreement in CCS values particularly for the 7^+ and 10^+ charge state is quite good with previous studies. We do note that the error for the 8^+ and 9^+ charge state is higher, which may be related to the fairly broad distribution of the protein at the charge states, which is fairly common for IMS data of intact proteins during potential unfolding events. (For further description of this phenomenon, see Dixit *et. al. Current Opinion in Chemical Biology*, **2018**, *42*, 93-100). ESI and IR-MALDESI CCS's on our instrument were quite similar as well, though a higher number of proteins and charge states (in addition to only aqueous solvent at physiological pH) could be examined in the future for a detailed comparison of activation.